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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:	WO 91/07682
G02B 21/00	A1	(43) International Publication Date:	30 May 1991 (30.05.91)

(21) International Application Number: PCT/GB90/01785

(22) International Filing Date: 20 November 1990 (20.11.90)

(30) Priority data:
8926435.2 22 November 1989 (22.11.89) GB

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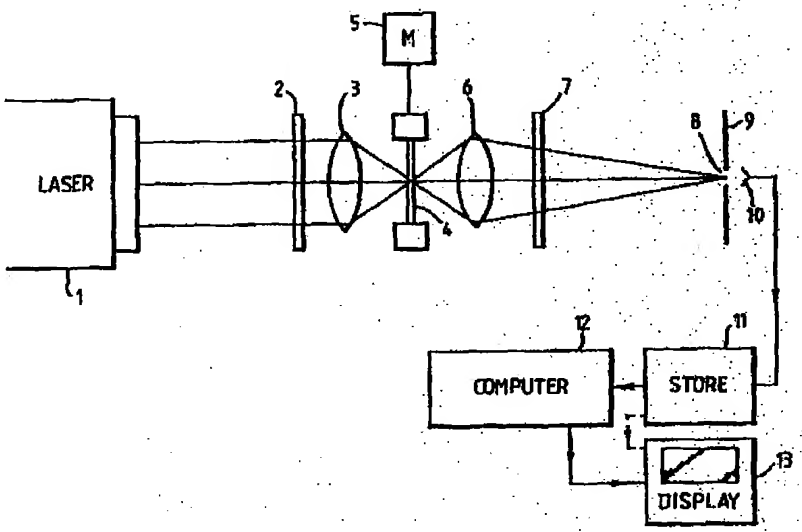
(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB, GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: PHASE MEASURING SCANNING OPTICAL MICROSCOPE



(57) Abstract

The invention provides for phase measurement in transmission and reflection scanning optical microscopes. In these microscopes a parallel beam of laser (1) light is focused onto a small portion of an object (4). Light from this small portion is imaged via a pinhole (8) onto a single detector (10). The image is built up by scanning the object and recording the output from the detector f r each object position. In this inventi n the phase image is recovered by subsequent processing of two or more amplitude images. One image is measured in the usual way; the other or others are recorded with suitable filters (2, 7) placed in the Fourier or output planes of both the illumination (3) and imaging (6) lenses. The two optical filters (2, 7) have amplitude weighting functions g(x, y) which preferably equal c. exp(ax + by), where a, b and c ar real constants.

According to this invention the phase image is recovered by subsequent processing of two r more amplitude images. One image is measured in the usual way; the other or others are recorded with suitable filters placed in the Fourier or output planes of both the illumination and imaging lenses. The terms light and optical are herein defined to include wavelengths either side of the visible wavelengths; the limits being determined by the availability of suitable light sources and detectors. The light source may be a laser, or bright light and pinhole.

According to this invention a scanning confocal microscope includes:-

an illuminating lens system for focusing light to illuminate a small portion of an object,

an imaging lens system for receiving light, which may be transmitted or reflected, from the small portion of the object and directing such received light through a pinhole aperture onto a detector or detectors,

means for scanning the object relative to the focused light,

means for storing the output of the detector or detectors to provide a display or displays of the scanned object,

characterised by:-

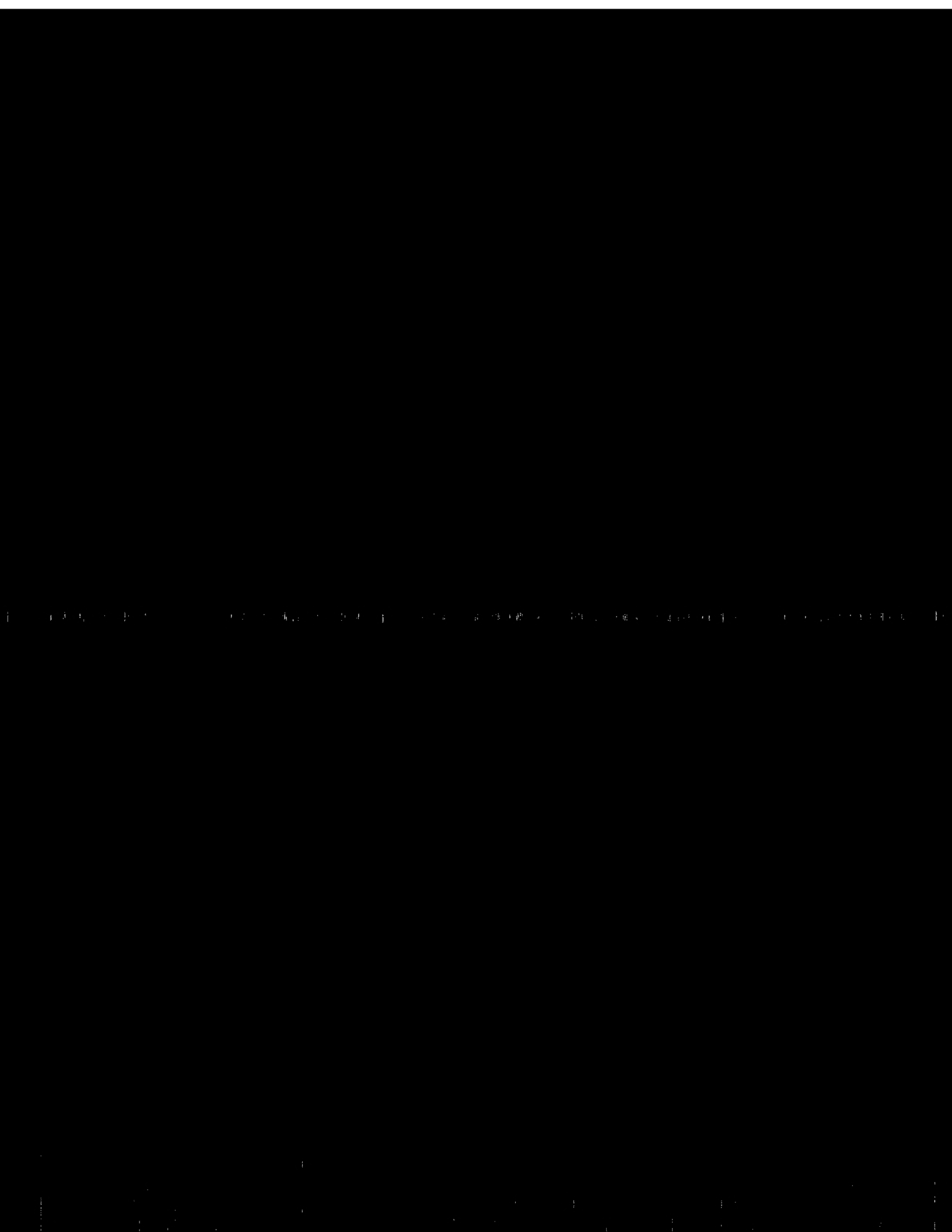
two movable optical filters having amplitude weighting functions $g(x,y)$ which can be positioned in the Fourier planes of the illuminating and imaging lens systems to modify the measured amplitude image, and

means for computing the phase image from detector data consisting of an amplitude image of the object measured without the filters in place and one or more amplitude images recorded with the filters in position in the Fourier planes.

The microscope of Figure 1 comprises a laser 1 whose parallel light output is directed through a first filter 2 onto an illuminating lens 3 of typically x100 magnification. The laser 1 may be HeCd laser perating at a wavelength of $.325\mu m$. the filter 2 is positioned so as to be in the lens Fourier plane ie. such that the complex amplitude in the object plane is the Fourier Transform of the filter function. Additional lens or lenses (not shown) may be used to achieve this. An object 4 to be measured is arranged at the focus of the illuminating lens 3 i.e., the object plane, and is mounted for movement across the optical axis of the microscope by a motor and drive 5. This allows scanning in an x-direction; a second motor and drive (not shown) orthogonal to the first motor allows scanning in the y-direction. Using a third motor and drive (not shown) images may be acquired at different positions in the z direction for 3-dimensional objects. An image lens 6 is arranged to receive light from the object and focus it through a second filter 7 onto a pinhole aperture 8 in a stop plate 9. The size of the pinhole is typically less than the size of the image of a point object formed in this plane. Light passing through the pinhole is measured by a detector 10 such as a photo-diode detector. Output from the detector 10 is to a store 11 including a storage medium such as a magnetic disc for subsequent output to a digital computer 12. The computer is used to implement an algorithm to retrieve the phase image from two or more amplitude images for subsequent display on a television monitor 13. Both object and image lenses 2, 6 have the same numerical aperture N, typically about 1.2 or more.

Apart from the filters 2,7 and the computer 12 this is similar to conventional scanning confocal microscopes (SCM). With such microscopes the amplitude image is sent directly from the store for display.

The purpose of the filters 2,7 is to multiply the illuminating and imaging Fourier planes by a filter or weighting function to enable the phase image to be recovered by subsequent processing. The preferable filter function $g(x,y)$ is $c.exp(ax + by)$, where a and b are of the order of $1/D$, where D is the diameter of the lens Fourier planes and c is chosen to keep $g(x,y)$ less than unity. In one typical example $D = 2mm, a = 0.5mm^{-1}, b = 0$ and $c = 0.7$.



The microscope shown in Figure 3 is similar to that of Figure 1 but operates on light reflected from an object 22. A laser 17 has its output directed via a beam expanding lens 18 and a filter 19 onto a refl cting beam splitter 20. From this beam splitter 20 light is focused by an image lens 21 onto the object 22. As before the object 22 is movable for scanning by a motor 23. Light reflected off the object 22 is collected by the image lens 21 arranged to receive light from the object and focus it through the beamsplitter 20 and a second filter 24 onto a pinhole aperture 25 in a stop plate 26. Light passing through the pinhole is measured by a detector 27 such as a photo-diode detector. Output from the detector 27 is to a store 28 including a storage medium such as a magnetic disc for subsequent output to a digital computer 29. The computer is used to implement an algorithm to retrieve the phase image from two or more intensity or amplitude images for subsequent display on a television monitor 30. Apart from the filters 19,24 and the computer 29 this is similar to conventional reflection scanning confocal microscopes (SCM). With such microscopes the intensity or amplitude image is sent directly from the store for display. Operation is as described for Figure 1.

The microscope shown in Figure 4 is similar to that of Figure one but contains an optical processing element 38 and additional lens 39 to enhance the resolution of the images obtained. The purpose of the optical processing plate 38 is to multiply the image plane by a predetermined function to improve the resolution obtained. Such an optical processing element 38 has an optical weighting function $m(x,y)$. For example, for a transmission or reflection microscope with square apertures and ideal lenses preferably the weighting function $m(x,y)$ is equal to $cos(2\pi Nx/\lambda).cos(2\pi Ny/\lambda)$ where N is the numerical aperture of the first and second lens systems and λ is wavelength. Alternatively a simple phase weighting function of constant amplitude but with the same phase as the above cosine function may be used ie.

$$m(x,y) = sign(cos(2\pi Nx/\lambda)).sign(cos(2\pi Ny/\lambda)).$$

The element has both a phase and amplitude weighting to incident light. The phase weighting may be achieved by etching grooves in the surface of a glass plate, whilst the amplitude weighting may be a variable density of photographic emulsion on the surface of a second glass plate. Both glass plates may be aligned and fixed together to form the optical processing element. Alternatively computer generated holographic plates may be used to achieve the same effect. For systems having a circular pupil the optical element has

detector 42 is to a store 43 including a storage medium such as a magnetic disc for subsequent output to a digital computer 44. The computer is used to implement an algorithm to retrieve the phase image from two or more amplitude images for subsequent display on a television monitor 45. Both object and image lenses 33,36 have the same numerical aperture N, typically about 1.2 or more. Apart from the filters 32,37 and the computer 44 this is similar to the amplitude measuring enhanced resolution scanning confocal microscope described in the co-pending patent application GB 89,13129, PCT/GB90/00868. With such microscopes the amplitude image is sent directly from the store for display. Operation is as described for Figure 1.

In a similar way the filters and computer may also be incorporated in the reflection enhanced resolution scanning confocal microscope described in the co-pending application GB 89,13129, PCT/GB90/00868. The microscope shown in Figure 5 is similar to that of Figure 4 but operates on light reflected from an object 55. The amplitude, or intensity, measuring version of this, Figure 5, enhanced resolution scanning microscope is described in the co-pending patent application GB 89,13129, PCT/GB90/00868. A laser 50 has its output directed via an object lens 51 and a first filter 52 onto a reflecting beam splitter 53. From this beam splitter 53 light is focussed by an image lens 54 onto the object 55. As before the object 55 is movable for scanning by a motor 56. Light reflected off the object 55 is focussed by the image lens 54 through the beamsplitter 53 and a second filter 57 onto an optical processing element 58 for processing as in Figure 4. Processed light is focussed by an additional lens 59 through a pinhole aperture 60 in a stop plate 61 and onto a detector 62. Output from the detector 62 is to a store 63 for subsequent output to a digital computer 64. The computer 64 is used to implement an algorithm to retrieve the phase image for display on a television monitor 65. The filters 52 and 57 are as described for Figure 4 and are in similar optical positions. Operation is as described for Figure 4.

With the filters in position $J_1(x) = J_0(x) \otimes G_1(x)$, where $G_1(x)$ is the amplitude diffraction pattern (Fourier Tansform) formed in the object plane 4 of the filter 2 and $J_2(x) = J_i(x) \otimes G_2(x)$, where $G_2(x)$ is the amplitude diffraction pattern (Fourier Tansform) formed in the image plane 9 of the filter 7, in which case, the modified amplitude falling on the detector ,$S'(\Delta x)$, is given by,

$$S'(\Delta x) = o(x) \otimes [(J_o(-x) \otimes G_1(-x)).(J_i(-x) \otimes G_2(-x))]. \qquad 7$$

For the case where both filters are of equal exponential form ie. $G_1(x) = G_2(x) = G(x)$ and where $G(x)$ is the Fourier transform of the filter function $g(x) = c.exp(ax)$; then using the special property of G(x) that for any functions $A(x)$ and $B(x)$,

$$A(x) \otimes G(x).B(x) \otimes G(x) = (A(x).B(x)) \otimes G(x) \qquad 8$$

equation (7) may be expressed in the form,

$$S'(\Delta x) = [o(x) \otimes (J_o(-x).J_i(-x))] \otimes G(-x). \qquad 9$$

Noting the form of equation (6) it may be seen that,

$$S'(\Delta x) = S(x) \otimes G(-x). \qquad 10$$

It may be shown (Opt. Acta. Vol 28, page 735, 1981) that measurements of the squared moduli (intensities) of two functions such as $|S(\Delta x)|^2$ and $|S'(\Delta x)|^2$ which are related by equation (10) are sufficient to determine the phase of $S(\Delta x)$. Figure 6 shows a flow chart of the computational steps of one of a number of different iterative or other methods that could be used to retrieve the phase of $S(\Delta x)$ from measurements of the two intensities $|S(\Delta x)|^2$ and $|S'(\Delta x)|^2$.

In this iterative method an initial estimate for the form of the image complex amplitude $S(x)$ is made and the iteration proceeds by alternately constraining the solution to be compatible with both the measured intensities $|S(\Delta x)|^2$ and $|S'(\Delta x)|^2$ and to be compatible with the known extent of the transfer function of the imaging system. The first stage is to input some estimate for the image complex amplitude, this may be a random function. This estimate

$S(\Delta x)$. There is a good agreement between the forms of the two phases. Th constant phase difference, which arises because constant phase terms have no influence on the measured intensity images, is of no practical importance.

Using the symbol ⊗ to denote convolution the form of th amplitude at the detector may be expressed as,

$$S_m(x) = o(x) \otimes [J_1(-x).(J_2(-x) \otimes m(x))]. \quad 15$$

With the filters removed $J_1 = J_o$ the amplitude point spread function of the illuminating lens 33 and $J_2 = J_i$ the amplitude point spread function of the imaging lens 36, in which case,

$$S_m(\Delta x) = o(x) \otimes [J_o(-x).(J_i(-x) \otimes m(x))] \quad 16$$

With the filters in position $J_1(x) = J_0(x) \otimes G_1(x)$, where $G_1(x)$ is the amplitude diffraction pattern (Fourier Tansform) formed in the object plane 34 of the filter 32 and $J_2(x) = J_i(x) \otimes G_2(x)$, where $G_2(x)$ is the amplitude diffraction pattern (Fourier Tansform) formed in the element plane 38 of the filter 37, in which case, the modified amplitude falling on the detector ,$S'_m(\Delta x)$, is given by,

$$S'_m(\Delta x) = o(x) \otimes [(J_o(-x) \otimes G_1(-x)).((J_i(-x) \otimes G_2(-x)) \otimes m(x))]. \quad 17$$

For the case where both filters are of equal exponential form ie. $G_1(x) = G_2(x) = G(x)$ and where $G(x)$ is the Fourier transform of the filter function $g(x) = c.exp(ax)$; then using the special property of G(x) given above in equation (8), equation (7) may be expressed in the form,

$$S'_m(\Delta x) = [o(x) \otimes (J_o(-x).(J_i(-x) \otimes m(x)))] \otimes G(-x). \quad 18$$

Noting the form of equation (6) it may be seen that,

$$S'_m(\Delta x) = S_m(x) \otimes G(-x). \quad 19$$

As before it may be shown (Opt. Acta. Vol 28, page 735, 1981) that measurements of the squared moduli (intensities) of two functions such as $|S_m(\Delta x)|^2$ and $|S'_m(\Delta x)|^2$ which are related by equation (19) are sufficient to determine the phase of $S_m(\Delta x)$. Again, the computational steps shown in figure 6 are of one of a number of different iterative or other methods that could be used to retrieve the phase of $S_m(\Delta x)$ from measurements of the two intensities $|S_m(\Delta x)|^2$ and $|S'_m(\Delta x)|^2$.

4. The microscope of claim 1 wherein at least one of the optical filters (2, 7, or 19, 24, or 32, 37, or 52, 57) is a plate of transparent material supporting a layer of variable transparency material.

5. The microscope of claim 1 wherein the layer of variable transparency material is a layer of variable density photographic emulsion.

6. The microscope of claim 1 wherein at least one of the optical filters (2, 7, or 19, 24, or 32, 37, or 52, 57) is a plate of optically absorbing material of varying thickness bonded to an inversely shaped plate of negligible absorption but similar refractive index to the absorbing material, the two plates forming a composite filter of constant phase retardation over all the composite filter.

7. The microscope of claim 6 wherein the composite filter has a uniform thickness and is formed of two plates of linearly varying thickness.

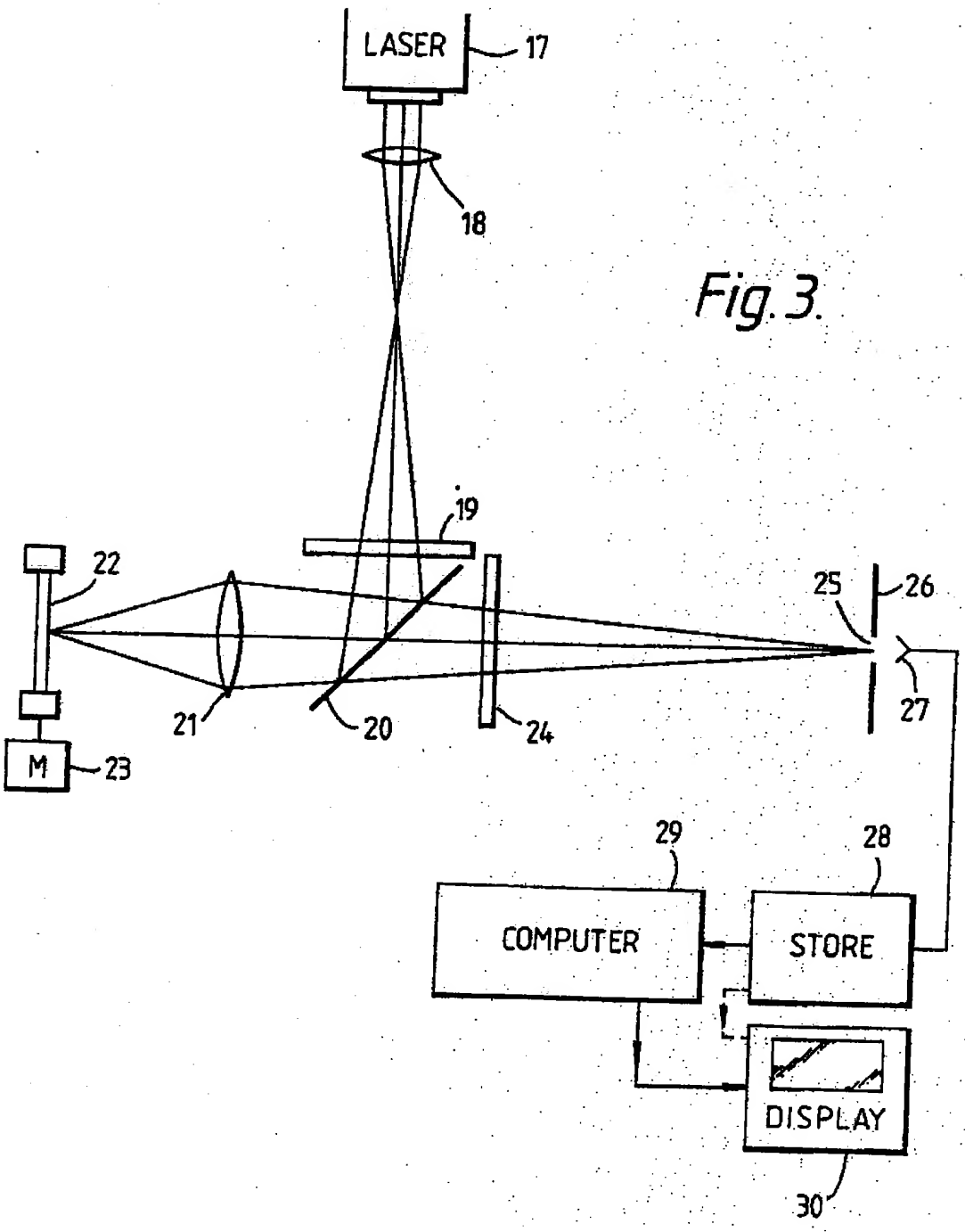

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Fig. 3.

Fig. 5.

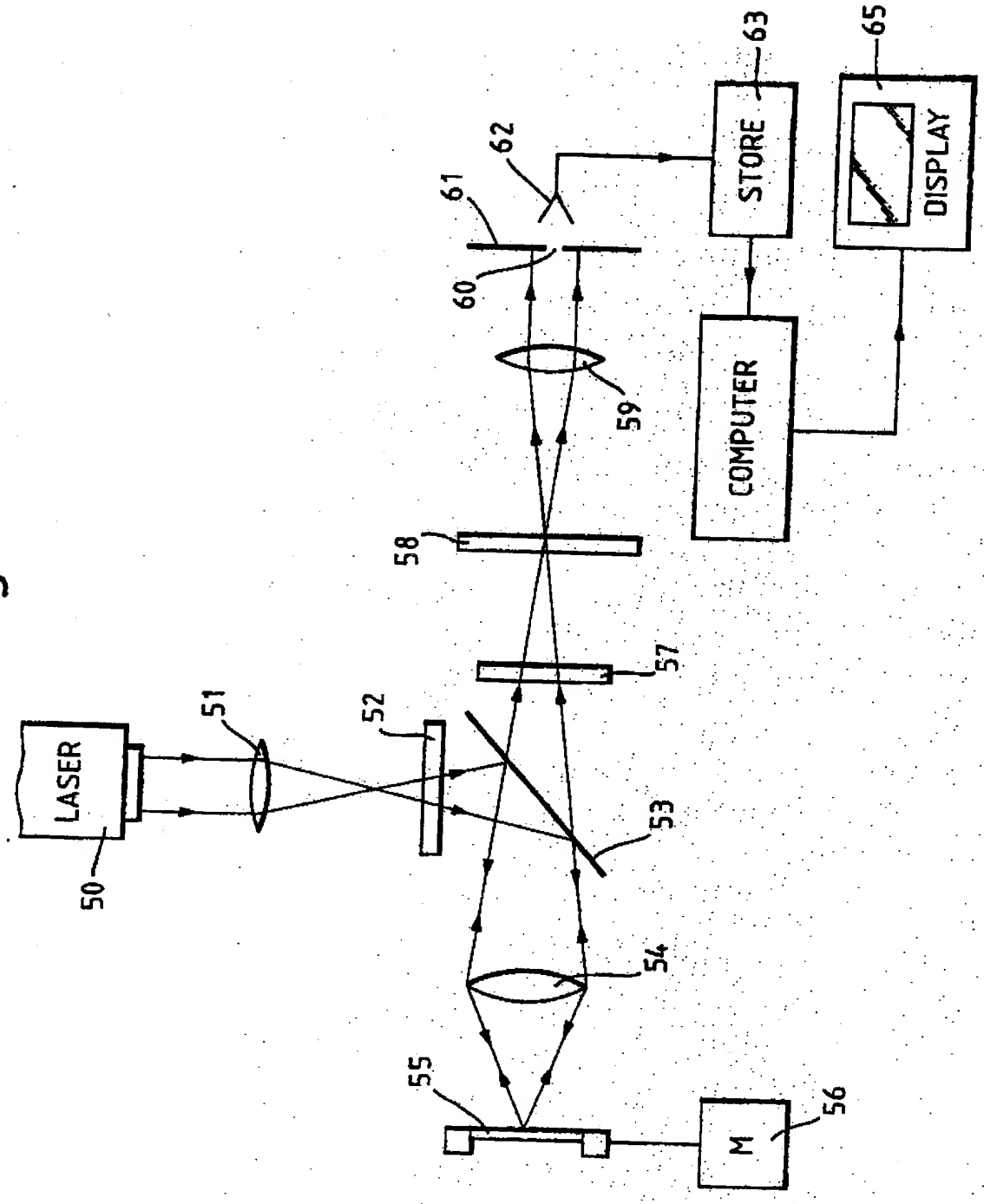
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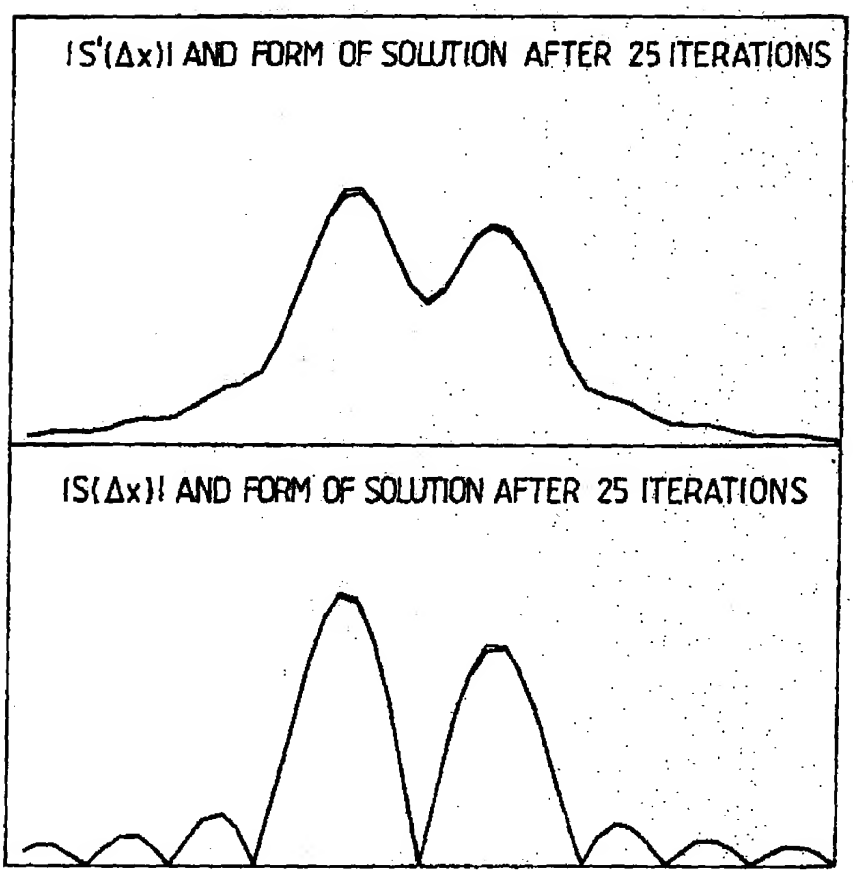
Fig.7.


|S'(Δx)| AND FORM OF SOLUTION AFTER 25 ITERATIONS
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